BIOLOGIA PLANTARUM (PRAHA)
25 (4) : 274—278, 1983

The Effect of Some Herbicides on Lactate Dehydrogenase Activity

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Abstract. The effect of herbicides N-(phosphonomethyl)glycine (glyphosate), 2-methyl-4-chlorophenoxyacetic acid (MCPA), 2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine (atrazine) and the respective commercial preparations "Round-up", "Aminex", and "Zeazin", on the activity of lactate dehydrogenase isolated from germinating soybean seeds and beef heart were compared. The main aim of the work was to compare the effect of the herbicides on lactate dehydrogenase isolated from animal and plant material. Simultaneously the effect of herbicides and the respective commercial preparations was compared. Substantial differences resulting from the two comparisons indicate differences in the structure of active centres of lactate dehydrogenase isolated from animal and plant material, and also a considerable effect of additives and surfactants in commercial preparations.

Contemporary agricultural production can hardly take place without the use of the herbicides. However, their application results in many problems. Herbicides and their residues remain in the soil and in plants, contaminate water, get into the tissues of farm animals and eventually also into man. They affect different metabolic systems of the above organisms to varying degrees (BERGMANNOVÁ and TAIMR 1973). Commercial herbicide preparations contain, besides the active substances, additives incl. surfactants which, however, represent the object of interest on relatively rare occasions. The aim of this paper was to contribute an understanding of the effects of some herbicides on lactate dehydrogenase isolated from beef heart and from soybean seedlings.

MATERIAL AND METHODS

Enzyme Isolation

Animal lactate dehydrogenase (lactate: NAD^+ oxidoreductase, EC 1.1.1.27) was isolated from beef heart. Plant lactate dehydrogenase from soybean (*Glycine max* L.) seeds which had germinated for 32 h in distilled water (BARTHOVÁ *et al.* 1980). The biological material was homogenized and extracted with 0.02 M Tris-acetate buffer, pH 8.6, containing 0.001 M

Received October 2, 1981; accepted November 25, 1982
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mercaptoethanol. The extracts obtained were fractionated with ammonium sulphate; the animal enzyme precipitated between 0 and 65% saturation and the plant enzyme between 30 and 40% saturation. The ammonium sulphate fractions were desalted by means of dialysis against a 0.001 M Tris-acetate buffer (pH 8.6) containing 0.001 M mercaptoethanol and concentrated by means of freeze-drying.

Herbicides

Three herbicides were studied:

N-(phosphomethyl)-glycine (glyphosate) in concentrations 0.73 and 1.34×10^{-2} M and the respective commercial preparation "Round up" in concentrations of active substance 1.0 and 1.83×10^{-2} M.

2-methyl-4-chlorophenoxyacetic acid (MCPA) in concentrations 5.0 and 9.2×10^{-3} M and the respective commercial preparation "Aminex" in concentrations of active substance 1.0 and 1.83×10^{-2} M.

2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine (atrazine) in concentrations 1.0 and 1.83×10^{-3} M and the respective commercial preparation "Zeazin" in concentrations of active substance 1.0 and 1.83×10^{-3} M.

The herbicides were dissolved in ethanol; when determining the enzyme activities, the corresponding ethanol concentration, *i.e.* 18%, was always used in control assay mixtures. The commercial preparations were dissolved in water and their concentrations related to the concentration of the active substance.

Determination of the Inhibition Constants

Lactate dehydrogenase activity was measured in the direction of lactate oxidation. The enzyme activity was determined on the basis of the rate of pyruvate production which reacted with 2,4-dinitrophenylhydrazine and formed red hydrazone. The amount of the resulting hydrazone was determined spectrophotometrically.

Determination of the inhibition constants of lactate dehydrogenase by the investigated inhibitors with NAD^+ was carried out for NAD^+ concentrations from 0.83 to 3.33×10^{-3} M at saturating lactate concentration, *i.e.* 0.2 M. Determination of the inhibition constants of lactate dehydrogenase by the investigated inhibitors with lactate was carried out for lactate concentrations from 0.83 to 3.33×10^{-2} at saturating NAD^+ concentration, *i.e.* 5×10^{-3} M. The assay mixture contained 0.1 M Tris-acetate buffer, pH 9.1, 2×10^{-4} M mercaptoethanol, and varying amounts of the inhibitors. The concentrations of the assay mixture components and experimental arrangement were devised so as to ensure linear reaction rate during the determination. The reaction was allowed to proceed for 15 min at 37 °C after adding the enzyme preparation to the assay mixture. Total volume of the assay mixture was 0.6 ml. The reaction was stopped by adding 0.25 ml of 0.1% 2,4-dinitrophenylhydrazine solution in 2 M HCl followed after 20 min at laboratory temperature by 2.5 ml of 0.4 M NaOH. Absorbance of the mixtures was determined spectrophotometrically at 505 nm after further 20 min using the spectrophotometer Spekol. Both animal and plant enzyme activities were determined under the same conditions to enable comparison of the activity values found.

Inhibition constants K_N and K_C were calculated from the measured values of reaction rates in dependence on substrate and inhibitor concentrations according to the relationship for mixed-type inhibition (KOTYK and HORÁK 1977):

$$v = \frac{V \times a}{K_m(1 + \frac{i}{K_C}) + a(1 + \frac{i}{K_N})},$$

where a = substrate concentration; i = inhibitor concentration; K_m = Michaelis constant for the respective substrate; K_C = dissociation constant of the complex enzyme-inhibitor which is determined from the slope of the dependence $1/v$ on $1/a$; K_N = the dissociation constant of the complex enzyme-substrate-inhibitor which is determined from the section cut on the ordinate by the dependence of $1/v$ on $1/a$; v = the initial reaction rate; V = maximum reaction rate.

The type of the inhibition was not estimated by plotting but numerically as follows:

The values of substrate concentrations denoted $a_1 \ldots a_6$ were chosen in such a way that $(1/a_n) - (1/a_{n+1})$ was approximately constant.

The respective absorbances measured were denoted $A_1 \ldots A_6$. Then

$$a_I = \frac{a_1 + a_2 + a_3}{3} \qquad a_{II} = \frac{a_4 + a_5 + a_6}{3}$$

$$A_I = \frac{A_1 + A_2 + A_3}{3} \qquad A_{II} = \frac{A_4 + A_5 + A_6}{3}$$

$$V = \frac{a_{II} - a_I}{A_I a_{II} - A_{II} a_I}$$

$$K_m = \frac{A_{II} - A_I}{A_I a_{II} - A_{II} a_I}$$

For an inhibited reaction, the respective values were denoted V_i and K_{mi}. The inhibition constants to be found were estimated from

$$K_N = \frac{i}{\frac{V}{V_i} - 1}$$

$$K_C = \frac{i}{\frac{K_{mi}}{K_m}\frac{V}{V_i} - 1}$$

The accuracy of the method is given only by the precision of the measurement and is therefore more accurate than plotting. The type of inhibition was estimated from the ratio K_C/K_N (KOTYK and HORÁK 1977). The variation coefficient for the estimation of inhibition constants was 2.77%.

TABLE 1

Inhibition of lactate dehydrogenase by herbicides. — Inhibition constants of lactate dehydrogenase towards NAD^+ as a substrate

Inhibitor	Animal LDH			Plant LDH		
	K_N [M]	K_C [M]	Type of inhibition	K_N [M]	K_C [M]	Type of inhibition
Round up	4×10^{-2}	4×10^{-2}	NC	5×10^{-2}	5×10^{-2}	NC
Glyphosate	$>5\times10^{-1}$	4×10^{-2}	C	$>5\times10^{-1}$	9×10^{-2}	C
Aminex	-2×10^{-2}	3×10^{-2}	*)	$>1\times10^{-1}$	5×10^{-3}	C
MCPA	2×10^{-2}	2×10^{-2}	NC	$>1\times10^{-1}$	4×10^{-3}	C
Zeazine	-1×10^{-2}	6×10^{-3}	*)	$>5\times10^{-2}$	$>5\times10^{-2}$	—
Atrazine	$>5\times10^{-2}$	1×10^{-2}	C	$>5\times10^{-2}$	$>5\times10^{-2}$	—

C: competitive inhibition; NC:noncompetitive inhibition; *): cannot be evaluated unambiguously; — : the enzyme was not inhibited.

RESULTS AND DISCUSSION

The inhibition constant values obtained and the types of inhibition are shown in Tables 1 and 2. Differences between the responses of the animal and the plant lactate dehydrogenases to the investigated herbicides were established.

The acid preparation MCPA shows a higher degree of inhibition with the plant enzyme than with the animal enzyme in contrast to the basic preparation atrazin which hardly inhibits the plant enzyme but markedly inhibits the animal enzyme. The inhibition of both the enzymes by the less acid preparation glyphosate does not differ substantially. It can be assumed that the acid preparations glyphosate and MCPA non-covalently interact in the reactive centre of the enzyme with basic residues of the amino acids histidine and arginine (HOLBROOK *et al.* 1975, BARTHOVÁ *et al.* 1980). The basic preparation atrazin may interact with acid residues of cysteine and tyrosine which are present in the active centre of the enzyme.

Differences between the effects of the commercial herbicide preparations and the proper active substances were found. The active substances inhibit lactate dehydrogenase in most cases competitively. Commercial preparations

TABLE 2

Inhibition of lactate dehydrogenase by herbicides. — Inhibition constants of lactate dehydrogenase towards lactate as a substrate

Inhibitor	Animal LDH			Plant LDH		
	K_N [M]	K_C [M]	Type of inhibition	K_N [M]	K_C [M]	Type of inhibition
Glyphosate	$>5\times10^{-1}$	5×10^{-2}	C	$>5\times10^{-1}$	4×10^{-2}	C
MCPA	$>5\times10^{-1}$	2×10^{-2}	C	$>1\times10^{-1}$	2×10^{-3}	C
Atrazine	$>5\times10^{-2}$	3×10^{-3}	C	-2×10^{-2}	-2×10^{-2}	A

C: competitive inhibition; A: activation.

inhibit lactate dehydrogenase mostly noncompetitively or in a mixed type and show a higher or the same degree of inhibition as the active substances. These differences are most likely caused by the additives which are present in the commercial preparations. An unusual type of inhibition is affected in the case of the animal lactate dehydrogenase by "Aminex" and "Zeazin". This may be the result of that fact that commercial preparations are chemical mixtures. These findings stress the importance of the study of commercial preparations and not only the chemically pure active substances. The differences in the inhibition of the animal and plant enzymes by commercial preparations are similar to the differences found in pure active substances.

Inhibitors presumably may also interact with substrates or coenzymes. However, the nature of differences in the effects of the investigated preparations on the animal and plant lactate dehydrogenase may rather be based on differences in the arrangement of the active centres of both enzymes, or on the different interaction of the substrate with the binding sites of the active centres resulting from it.

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